To learn practical skills in conducting viral disease challenge techniques in Penaeid prawn species using white-spot syndrome virus (WSSV) as a model virus to be conducted at Shrimp Biotechnology Business Unit (BIOTEC), Pathumthani, Thailand.

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Project No.

2013/718

This project was conducted by the University of Tasmania

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The Australian Seafood CRC is established and supported under the Australian Government's Cooperative Research Centres Program. Other investors in the CRC are the Fisheries Research and Development Corporation, Seafood CRC company members, and supporting participants.

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Australian Government
Fisheries Research and Development Corporation

An Australian Government Initiative
CRC AUSTRALIA

ISBN: 978-0-9752258-9-9

NON-TECHNICAL SUMMARY

PROJECT NO:

2013/718

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(PROJECT) OBJECTIVES OF RESEARCH TRAVEL GRANT/ INDUSTRY BURSARY

To learn practical skills in conducting viral disease challenge techniques in Penaeid prawn species using white-spot syndrome virus (WSSV) as a model virus to be conducted at Shrimp Biotechnology Business Unit (BIOTEC), Pathumthani, Thailand.

NON TECHNICAL SUMMARY:

I travelled to Thailand where I helped conduct a commercial growth and disease challenge trial at Shrimp Biotechnology Business Unit (SBBU), a commercial department of BIOTEC and the National Science and Technology Development Agency (NSTDA).

This consisted of feeding 5 different feeds (1 x control, 3 x immunostimulants, 1 x commercial reference) for 38 days to obtain growth performance, feed intake, survival and immune response data. During this time I worked with NSTDA employees conducting routine duties such as feeding the prawns four times daily, water quality analysis and tank cleaning. At the conclusion of the feeding trial we weighed every prawn and sampled blood (n = 21 / feed treatment) for immune response measures; total blood cell count (THC), granular blood cell count (DHC) and immune cascade enzyme (PO Activity).

After the growth trial we conducted a viral disease immune response challenge, using White Spot Syndrome Virus (WSSV) as a model virus. In this trial we obtained WSSV from naïve prawns which were infected from WSSV diseased prawns isolated within a mesh cage. The viral inoculum was isolated and then quantified using molecular techniques. From preliminary studies conducted prior to this experiment the lethal dose, where 50% of prawns

die in 7 days was calculated and used as the dose for the current experiment. Forty prawns from the control and each immunostimulant feed treatments were divided into 4 additional treatments; intramuscular injection (PBS), intramuscular injection (WSSV), reverse gavage (PBS) and reverse gavage (WSSV). The PBS treatments were the controls were 100% survival rates are expected, while WSSV treatments are the disease treatments where mortality is expected. Prawns were inoculated using these methods and then sampled 48 h after infection for blood immune responses (THC, DHC & PO activity) and histopathology.

The final experiment was the WSSV disease challenge where prawns were inoculated with either PBS or WSSV and mortality/survival calculated over 21 d, to determine whether a particular feed treatment and or inoculation provides better survival to WSSV. This experiment was conducted similar to the last using more prawns, where 120 prawns per feed treatment were divided into the same 4 method treatments and inoculated, mortality was calculated daily to determine a survival curve which was analysed for comparisons between feed and inoculation method. Pleopods were obtained from three dead prawns of each tank and were individually quantified for WSSV load, to help further determine if one feed treatment provided more resistance to the WSSV. The quantification of virus was conducted using molecular techniques (real-time PCR).

I visited a commercial shrimp farm (*Litopenaeus vannamei*) located in the Phetchaburi province of Thailand. The farm is located in an area where isolated viral disease (Yellow Head Virus (YHV) and Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) outbreaks occur every few weeks, although the farm has a stringent biosecurity and husbandry program and subsequently been viral free for at least 3 years. On arrival at the farm Vehicles are sterilized by a drive-through bath and spray system containing an iodine solution. Before entering the ponds and water treatment canals, another vehicle bath is used. Each pond has specific gumboots, dip-nets to be used and sterilized on entering and exiting the pond. All ponds including water storage ponds are covered with a very fine mosquito mesh. The Manager said this is one reason that we don't suffer from YHV, as it is common for seagulls and other birds to drop infected shrimp from farms not covered with mesh. While the cost of infrastructure and netting to cover each pond is substantial it is far cheaper than losing your stock and then trying to eradicate the virus from the farm. On my way to the farm I noticed that bird netting is very common on larger farms, while small farms of 2-3 ponds are left un-covered. Incoming water for ponds is treated up to 3 times with different chemicals, including bleach to remove any algae, larval animals, bacteria and viruses. Due to the risk of introducing pathogens when pumping water into the farm, all effluent water is treated and re-used as much as possible.

OUTCOMES ACHIEVED TO DATE

Conducted growth trial
Conducted WSSV immune response trial
Visited to commercial shrimp farm
Tutorial Presentation of this research to the Aquatic Animal Health 3rd year students and Masters students, University of Tasmania.
Practical laboratory session for 3rd Aquatic Animal Health students using practical skills and theoretical knowledge obtained from my visit to SBBU and BIOTEC.

(PROJECT) OUTPUTS DEVELOPED AS RESULT OF TRAVEL GRANT/ INDUSTRY BURSARY:

I was able to enhance my knowledge and obtain practical skills of viral disease challenging, using WSSV as a model, which is considered the bench-mark pathogen for testing immunostimulants and other bioactive ingredients. The methods learnt to prepare and conduct the viral disease challenge were applied to prawns which had been feeding on experimental used throughout my PhD research, to further explain potential benefits of the tested immunostimulants on viral pathogens exotic to Australia.

The travel grant also allowed professional development opportunities, where I worked with World renowned scientists at the NSTDA and will also allow for future collaborations.

From my visit to a commercial prawn farm I was able to get first-hand experience of the current problems that Thailand farmers experience and learn how they overcome the problems of viral outbreaks to ensure they stay competitive in the shrimp farming industry.

Moreover, the knowledge and skills attained from my visit to Thailand, which was made possible by the CRC travel funding, allowed me to present the data obtained from this research to students studying Aquatic Animal Health at the University of Tasmania, in the form of a tutorial and practical laboratory session.

Furthermore, the data and results from obtained from the research conducted in Thailand will make part of my final PhD thesis, which will be made available to collaborative partners of my PhD (Ridley Aquafeeds, Marinova Pty Ltd, Australian Prawn Farmers Association and the CRC).

ABOUT THE PROJECT/ACTIVITY

BACKGROUND AND NEED

The prawn aquaculture industry including the aqua feeds industry is continuously evolving to ensure the production output meets the demand by consumers. Due to the nature of commercial prawn farming, constraints within the industry such as disease management will always pose problems from managing endemic diseases; such as gill-associated virus in Australia, to exotic diseases including Yellow Head Virus and White Spot Syndrome Virus. My PhD project aims to optimise prawn nutrition for growth performance under sub-optimal conditions. In many instances the component of sub-optimal conditions (stressor) is necessary to initiate a disease outbreak in an acute expression. Therefore when testing new ingredients such as immunostimulants, it is beneficial to use a stressor/pathogen which can cause considerable stress or mortality to assess how effective the product is. White Spot Syndrome Virus has been used extensively as a model pathogen for testing the efficacy of immunostimulants, probiotics, prebiotics etc. Another important factor is the method of inoculation, how the virus is incorporated into the animal. The two most common methods include intramuscular injection (IM) and immersion. However, when considering natural infections in commercial culture, prawns are not injected with viruses, they do to some extent immerse in the virus at very dilute concentrations. The IM method bypasses the gut and the immersion technique does not take into account the viral dose taken up by the prawn. Therefore, this research compared the traditional method used in the literature with a novel technique of reverse gavage (anal intubation); although not natural the method uses a known concentration of virus. This is important when assessing immunostimulants which may provide protection from viruses within the gut, by stopping viral adsorption so viruses cannot multiple. This study will provide beneficial for feed producers when considering methods for testing novel immunostimulants or bioactive ingredients.

The need to conduct this research outside of Australia is important due to the model virus being of ‘exotic’ nature and importing the virus is not possible. To be able to test the ingredients in a disease challenge experiment and to learn to skills associated with such an experiment it was necessary to find a commercial laboratory overseas which can provide the infrastructure and expertise required. Depending upon the results (pending) this method of inoculation may be a preferred method used by the feed industry for testing new feeds, or to compare with the IM method.

RESULTS

I have gained extensive knowledge of how to conduct a viral disease challenge using WSSV. Furthermore the practical skills/techniques which I have learnt are important for this research, which is part of my PhD project. Once the current experiment is concluded and results

collated it will be possible to make more statements regarding the efficacy of the reverse gavage method for assessing immunostimulants in prawn feeds. Further statements regarding the efficacy of immunostimulants will be made and the potential for use in prawn feeds in Australia.

I gained extensive knowledge of the current factors faced by some Thailand prawn farmers, regarding management strategies for disease-free production. From our discussions, research into new/existing immunostimulant products will play an important part of prawn production for many years to come in Thailand.

INDUSTRY IMPACT

PROJECT OUTCOMES (THAT INITIATED CHANGE IN INDUSTRY)

SUMMARY OF CHANGE IN INDUSTRY

Immediate changes relevant for the Australian prawn farming feed producers or businesses, including prawn farmers are limited at this stage. The reason is due to pending results, as the final experiment of three experiments is currently in progress.

The laboratory exchange comprised of conducting routine husbandry and experimental techniques, from conducting three individual experiments. The first experiment was a feeding trial, which concluded on the 9th of September. Following this was a White Spot Syndrome Virus (WSSV) disease challenge where immune parameters (total haemocyte counts (THC), differential haemocyte counts (DHC) and phenoloxidase activity (PO activity) were analysed 48 h after inoculation with WSSV. Whole body tissue histology was also sampled at 48 h for comparing viral associated pathology using the two inoculation methods, intramuscular injection (IM) and reverse gavage (RG). The final experiment (WSSV disease challenge) which started on the 25th of September will continue up to three weeks, were survival rates will be measured; duration of this experiment will depend on the mortality rate. Also, until results can be properly analysed any growth promoting factors from this research cannot be made.

WHAT FUTURE AND ONGOING CHANGES ARE EXPECTED?

The main objectives of the research was to obtain new knowledge of the effectiveness of commercial immunostimulants and to obtain practical skills in conducting viral disease experiments, using traditional methods IM in association with a new novel technique RG. Traditionally IM has been conducted to assess whether particular immunostimulants can provide increased resistance to commercially important diseases, included WSSV, or to determine if prawn stock have specific pathogen free status, free of a particular disease. The problem associated with the IM method is that it bypasses the natural route of infection. This is problematic when assessing immunostimulants/bioactive ingredients as the gut and intestine is bypassed, therefore eliminating potential benefits of disease resistance. Once the

experiment is concluded and the results analysed I will be able to comment on the future use of RG and how it can be implemented in disease trials.

WHAT BARRIERS ARE THERE FOR CHANGES TO OCCUR?

Currently the most common method used within the literature for inoculating prawns in a disease challenge is by IM. Therefore, comparisons are made between literature using the same methods to determine if a particular feed ingredient is better than another. It is for this reason that comparisons between new 'novel' and traditional methods will be very difficult or impossible. Further research into RG will be required to determine the limits of the technique.

IF NOT ALREADY HAPPENING, WHEN WILL THE CHANGES OCCUR?

This research is supported and partially funded by Ridley Aquafeeds, and the tested ingredients and methods are of particular interest to Ridley Aquafeeds. Also Ridley Aquafeeds conduct research at SBBU, so results will be comparable to past research conducted there. If the pending results show potential to use RG in future research then this may be used within the next 12 months.

WHAT IS THE LIKELIHOOD THAT THESE CHANGES WILL OCCUR?

This research has direct industry involvement, from local feed producers to prawn farmers. This research aimed to validate the RG technique by assessing immunostimulant products; any demonstrated improvements over traditional methods will be highly desirable and applicable to nutrition-health related research. Furthermore, there is no additional cost associated with the RG method.

WHAT BARRIERS ARE THERE TO DOPTION OF THESE CHANGES AND WHAT ACTION COULD BE TAKEN TO OVERCOME THESE?

The RG technique may require further validation and optimisation.

COMMUNICATION OF PROJECT/EXTENSION ACTIVITIES

WHAT IS THE OUTPUT THAT NEEDS TO BE COMMUNICATED?

Skills and knowledge attained from conducting the experiments at SBBU and farm visit has been communicated through teaching undergraduate degree students, through tutorial and practical class. Results obtained will be communicated at the completion of my PhD and at annual seminars to the University.

WHO IS/ARE THE TARGET AUDIENCE/S?

Target audiences are researchers, staff and students at the University of Tasmania who are involved in my project, or who attend seminars as part of my PhD project. Annual reports of my research results are sent/or delivered to the APFA, Ridley Aquafeeds and Marinova Pty Ltd, and my PhD thesis will be sent to all collaborators at the conclusion of my PhD.

WHAT ARE THE KEY MESSAGES?

At times it is difficult to learn the necessary skills locally, this may be due to lack of expertise or restrictions due to limited or no access to pathogens. Therefore, it is necessary at times to travel abroad to obtain the skills and experience required to conduct your research.

WHAT IS THE CALL TO ACTION?

To consider new techniques for assessing feed ingredients

COMMUNICATION CHANNELS

(How can these messages be communicated and by who?):

Channel	*Who by*	*When*
Communication with researchers in crustacean health and nutrition	Daniel Pountney	Ongoing
Demonstrating to my colleagues and peers new techniques and skills gained from conducting disease challenges	Daniel Pountney	Already conducted and ongoing
Incorporating new techniques, skills and assays in my research	Daniel Pountney	Submission of thesis

LESSONS LEARNED AND RECOMMENDED IMPROVEMENTS

WHAT IS YOUR FEEDBACK?

Conducting research abroad has some limitations due to language barriers and cultural differences. It is important that you stand firm when important decisions are made and be able to compromise a little for less important matters. To be able to value-add your research

with commercial farm visits is very important and knowledge gained is very valuable. Be prepared to allow enough time as planned lab or farm visits may not always stay on schedule.

FURTHER ACTION REQUIRED IN REGARDS TO COMMERCIALISATION?

Intellectual property is governed by the contract between the Seafood CRC and the University of Tasmania for this project.

ACKNOWLEDGEMENTS

I would like to acknowledge the Seafood CRC for the travel grant.